Institute Report No. 346

Dermal Sensitization Potential of Trimethylolethane Trinitrate (TMETN) in Guinea Pigs

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and
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DIVISION OF TOXICOLOGY

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ABSTRACT

Trimethylolethane trinitrate (TMETN) was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence of TMETN-induced sensitization was obtained in the study.

Key Words: Dermal Sensitization, Mammalian Toxicology, Trimethylolethane Trinitrate, TMETN, Buehler Test, Guinea Pigs, Propellant

PREFACE

TYPE REPORT: Dermal Sensitization GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 84042

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Yvonne C. LeTellier, BS

CO-INVESTIGATOR: Larry D. Brown, DVM, LTC, VC, Diplomate,
American College of Veterinary Preventive Medicine,
American Board of Toxicology.

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Trimethylolethane Trinitrate (TMETN)

INCLUSIVE STUDY DATES: 15 January - 1 March 1985

OBJECTIVE:

The objective of the study was to evaluate the dermal sensitization potential of trimethylolethane trinitrate in guinea pigs.

ACKNOWLEDGMENTS

SP4 Paul B. Simboli, BS, and Gerald F.S. Hiatt, PhD, provided technical assistance. SP4 Scott Schwebe provided animal care and managed the facilities. Colleen Kamiyama and Ann Wilkinson provided office management during the performance of the study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84042 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 11 July 89
DON W. KORTE, JR., PhD / DATE
LTC, MSC
Study Director

Yvonne C. LeTellier 11 Jul 1989
YVONNE C. LETELLIER, BS / DATE
DAC
Principal Investigator

Larry D. Brown 3 July 1989
LARRY D. BROWN, DVM / DATE
LTC, VC
Co-Investigator

Conrad R. Wheeler 10 July 89
CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

5 July 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84Ø42

1. This is to certify that the protocol for LAIR GLP Study 84Ø42 was reviewed on 15 October 1984.

2. The institute report entitled "Dermal Sensitization Potential of Trimethylolethane Trinitrate (TEMTN) in Guinea Pigs," Toxicology Series 139, was audited on 27 June 1989.

Carolyn M Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

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INTRODUCTION

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the dermal sensitization potential of trimethylolethane trinitrate (TMETN) in guinea pigs.

MATERIALS

Test Substance

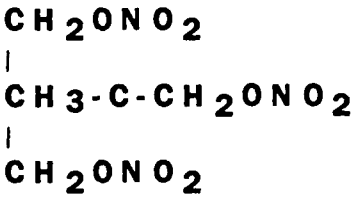
Chemical Name: Trimethylolethane trinitrate (TMETN)

Chemical Abstracts Service Registry No.: 3032-55-1

LAIR Code Number: TA35

Physical State: Liquid

Chemical Structure:



Molecular Formula: $C_5H_9N_3O_9$

Source: Naval Ordnance Station
Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle for Test Substance

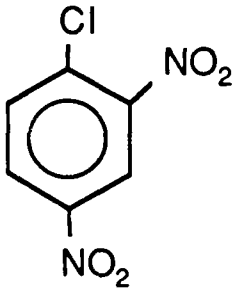
A pilot study indicated that neat TMETN (100%) was not a dermal irritant in the guinea pig. Therefore, neat TMETN was used in this study and no vehicle or vehicle control group of animals was necessary.

Positive Control

Chemical Name: Dinitrochlorobenzene (DNCB)

Chemical Abstracts Service Registry No.: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Other positive control substance information is presented in Appendix A.

Vehicle for Positive Control

A 0.1% solution of DNCB was prepared weekly, on 4 March, 11 March, and 1 April 1985. The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485, Exp. Date 1991) was obtained from Certified Laboratories, Inc., (Philadelphia, PA). Sterile, isotonic saline (lot number 7C950X0, Exp. Date Oct 1985) was obtained from Travenol Laboratories, Inc., Deerfield, IL.

Animal Data

Male albino guinea pigs, Hartley strain (Charles River Breeding Laboratories, Wilmington, MA), from a shipment received on 15 January 1985 were assigned to this study. They were identified individually with ear tags. Two animals (85E0056, 85E0067) were selected for quality control necropsy evaluation on receipt. Animal weights on the day following receipt ranged from 178 to 232 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs assigned to this study were caged individually in stainless steel, wire mesh cages in racks equipped with automatically flushing dump tanks. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow® Diet 5026 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. Temperature within the animal room was maintained in the range from 21.7 to 29.4°C. Relative humidity was maintained in the range of 26 to 50%. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-82 "Buehler Dermal Sensitization Test" (2) and EPA guidelines (3).

Group Assignment/Acclimation

The guinea pigs were quarantined for 15 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Fifteen animals were assigned to each of three groups by a stratified randomization technique based on their body weights.

Dose Levels

Three animal groups comprise the basis for this report. Dermal sensitization potential was evaluated in a test group receiving three weekly induction doses of 100% trimethylolethane trinitrate and, after a two-week delay, a challenge dose at the same concentration. Dinitrochlorobenzene, a known potent sensitizing agent (4), was applied to another group, at a 0.1% concentration, as a positive control. A negative control group received 100% trimethylolethane trinitrate only on the day of challenge dosing.

Compound Preparation

TMETN was received as a liquid in 10% ethanol. Rotoevaporation was performed to remove the ethanol, resulting in neat TMETN. TMETN was used neat (undiluted) in the study. The dinitrochlorobenzene (DNCB) dosing solution was prepared by first adding 30 mg DNCB to 1.0 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (5-7) to mimic the repeated-insult patch test for humans. Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish

between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization, a negative control group was added which received only the challenge dose.

During the induction phase, the test and positive control groups were dosed with 0.5 ml of the appropriate compound/suspension applied topically under a 2.5-cm^2 gauze patch. This procedure was performed for three consecutive weeks (29 Jan and 5, 12 Feb 85). Twenty-four hours before each dosing, a 7.6-cm^2 area on the left flank of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT). The patch was taped with Blenderm® hypoallergenic surgical tape (3M Corp., St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vetrap® (3M Corp., St. Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was gently wiped of any excess compound using a saline-moistened gause and the site was marked for scoring.

Animals were challenged two weeks (26 Feb) following the third induction dose. Test group and positive control group animals received two 0.5-ml doses each of TMETN or DNCB, respectively, one applied to the old site on the left flank and the other to a new site on the right flank. Negative control animals received only a single 0.5-ml dose of TMETN, applied to the left flank. Procedures for clipping, shaving, and wrapping and the exposure period remained the same.

In Buehler's procedure, skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24, 48, and 72 hours after each induction dose as well as 24, 48, and 72 hours after the challenge dose. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema), and 3 (marked erythema). Results are expressed in

terms of both incidence (the number of animals showing responses of 1 or greater at either 24, 48, or 72 hours) and severity (the sum of the test scores divided by the number of animals tested). Results from the left flank are compared with right flank and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose. For consistency with induction procedures, this step was replaced by clipping the animals.

The animals were observed daily for clinical signs and weight gain was monitored during the study. At the conclusion of the study, a necropsy was performed on each animal. A historical listing of study events appears in Appendix C.

Changes/Deviations

This study was conducted in accordance with the protocol and applicable amendments.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Experimental

Table 1 summarizes the incidence of reactions 24, 48, and 72 hours after each dose. No reaction was observed in response to trimethylolethane trinitrate after any of the induction doses or the challenge dose. This lack of response is reflected in Table 2 which depicts the severity of skin reactions.

Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. For trimethylolethane trinitrate no responses were obtained; therefore, severity scores were zero at all times.

Positive Control

Dinitrochlorobenzene produced a marked response at all time points after the first induction dose (Table 1). Between 67% and 100% of the DNCB-treated animals exhibited a response 24 hours following the second or third induction and challenge doses. These reactions persisted, yielding scorable effects in 46-100% of the animals at 48 hours after dosing and 33-100% of the animals at 72 hours after dosing. Severity scores for these responses to DNCB ranged from 0.1 to 1.3 at the 24-hour scoring period (Table 2). The highest score, 1.3, was observed in response to the second induction dose. By 48 hours the reactions had subsided slightly; consequently, the severity range decreased to between 0.1 and 1.2. At 72 hours the reactions diminished further to a range of 0 to 1.1.

Negative Control

No response was observed in the negative control (challenge dose of TMETN) group. Individual 24-hour, 48-hour, and 72-hour dermal scores for all animals appear, by group, in Appendix D.

Clinical Signs

All animals were healthy and gained weight during the study. Individual body weight data are presented in Appendix E.

Pathology Findings

A necropsy was performed on all study animals. Minimal to moderate hepatic necrosis was identified in almost all test animals at study termination. This is a commonly observed incidental finding in guinea pigs. The complete pathology report is presented in Appendix F.

TABLE 1: Incidences of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
		24 Hours			
TMETN	0/15	0/15	0/15	0/15	0/15
Negative Control*	—	—	—	0/15	—
DNCB	1/15	10/15	13/15	15/15	11/15
		48 Hours			
TMETN	0/15	0/15	0/15	0/15	0/15
Negative Control*	—	—	—	0/15	—
DNCB	2/15	7/15	14/15	15/15	12/15
		72 Hours			
TMETN	0/15	0/15	0/15	0/15	0/15
Negative Control*	—	—	—	0/15	—
DNCB	0/15	5/15	8/15	15/15	10/15

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 2: Severity of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
		24 Hours			
TMETN	0.0	0.0	0.0	0.0	0.0
Negative Control*	—	—	—	0.0	—
DNCB	0.1	0.7	0.9	1.3	0.8
		48 Hours			
TMETN	0.0	0.0	0.0	0.0	0.0
Negative Control*	—	—	—	0.0	—
DNCB	0.1	0.5	0.9	1.2	0.7
		72 Hours			
TMETN	0.0	0.0	0.0	0.0	0.0
Negative Control*	—	—	—	0.0	—
DNCB	0.0	0.3	0.5	0.7	1.1

*The Negative Control Group received only a challenge dose of the test compound.

DISCUSSION

Dermal Irritation and Sensitization

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference between irritation and sensitization is the mechanism responsible for this inflammation. Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis. Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical (8). Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. Although both types of reactions can appear grossly similar in experimental animals and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, whereas sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, whereas only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction (a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, whereas the reaction to a sensitizer often increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration, whereas a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to

an irritant may spread beyond the area of contact, whereas sensitization reactions are usually circumscribed.

The features of irritation and sensitization have been used to establish guidelines for differentiation between the two (5-8). In evaluating a dermal sensitization study it is recommended that the results from a challenge dose in the experimental group (sensitization) be compared with those for the negative control group (irritation) in accordance with the following criteria:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 hours, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, the intensity/severity of inflammation often is greater at 72 to 96 than at 24 to 48 hours.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy potential is evaluated by the method of Draize et al (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory reaction is graded. Evaluation of sensitizing potential is accomplished by repeated application, at lower non-irritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow the immune system to elaborate and increase its specific response to the chemical. A challenge dose is then given, and the resulting inflammatory response is graded. Analysis of the incidence, severity, and timing of the response to the challenge dose estimates the sensitizing potential of the study compound.

Trimethylolethane trinitrate

Trimethylolethane trinitrate (TMETN) was evaluated for its ability to elicit a delayed-hypersensitivity or cellular immunologic reaction via contact with the skin. TMETN produced no response indicative of the potential to elicit dermal sensitization when evaluated according to the method of Buehler and Griffith (5-7).

Sensitization produced by TMETN would have been detected by this study. A hypersensitivity-type response was reliably elicited by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously within the Institute (10). Although DNCB is capable of producing primary irritation, the characteristics of the responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also, the response to DNCB was observed primarily after two or more exposures.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than does man, this result does not guarantee that TMETN will not sensitize humans. However, it does indicate that TMETN is unlikely to sensitize humans and its potential is low enough to permit its evaluation in man.

CONCLUSION

Trimethylolethane trinitrate (TMETN) possesses minimal sensitizing potential, as it did not induce a dermal sensitization reaction under conditions of this study.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethan trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA 127846.

2. Buehler dermal sensitization test. LAIR Standard Operating Procedure OP-STX-82. Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.

3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Dermal sensitization. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

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Appendix A: CHEMICAL DATA

Chemical Name: 1,3-Propanediol, 2-methyl-2[(nitrooxy)methyl]-dinitrate (ester)

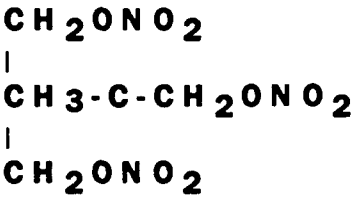
Other Names: 1,3-Propanediol-2-(hydroxymethyl)-2-methyl-, trinitrate; 1,1,1-Trimethylolethane trinitrate(TMETN), Metriol trinitrate (MTN); Nitropentaglycerin

Lot Number: 53-84A

Chemical Abstracts Service Registry No.: 3032-55-1

LAIR Code No.: TA35

Structural Formula:



Molecular Formula: $C_5H_9N_3O_9$

Molecular Weight: 255.15

Physical State: Light brown oil

Melting Point: -3° [1,2]

Compound Density: 1.47 g/cm [1,2]

Source: Naval Ordnance Station, Indian Head, MD, 20640

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[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p 17.

[2] Lindner V. Properties of explosive aliphatic nitrate esters. Table 5. In: Grayson M., exec. ed. Kirk-Othmer Encyclopedia of Chemical Technology. Volume 9. 3rd ed. New York: John Wiley and Sons, Inc., 1980:573.

Appendix A (cont.): CHEMICAL DATA

Analytical Data: Ultraviolet (UV) spectra were obtained using a Hitachi 110-A Spectrophotometer (Hitachi Instruments, Inc., Mountain View, CA), infrared spectra (IR) were obtained with a Perkin-Elmer Model 457 Infra-red Spectrophotometer (Perkin-Elmer, Norwalk, CT) and nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT-80 NMR (Varian, Palo Alto, CA) using tetramethylsilane as an internal standard. Chromatographic analysis was performed using a 1090B HPLC with diode array detector (Hewlett-Packard, Santa Clara, CA) and a Brownlee RP-18 Spheri-5 Column, 4.6 x 250 mm (Brownlee Labs, Inc., Santa Clara, CA). The following conditions were employed for the HPLC assay: solvent system, 70% methanol, 30% water; flow rate, 0.9 ml/min; detector wavelength, 215 nm; oven temperature, 50°C.

UV Spectrum: For UV analysis TMETN was dissolved in acetonitrile. UV absorbance begins at approximately 240 nm and increases with decreasing wavelength.[3] No absorption peak was observed. IR (KBr windows): 2900, 1645 (asymmetric stretch of NO group, 1470, 1375, 1280 (symmetric stretch of NO_2 group), 990, 860, and 755 cm.[4] ^1H NMR (CDCl, 80 MHz): d 1.22 (S, 3H, CH_3), 4.44 (S, 6H, $-CH_2-$).[5] TMETN subjected to HPLC analysis eluted as two peaks with retention times of 5.5-5.6 and 12.5 min.[6] Based on integration of peak areas, the first peak represented 98% of the sample. The second peak was not identified. No decomposition of TMETN was detected by HPLC after storage of TMETN (neat or in ethanol) for a period of nine weeks.[7]

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[3] Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 51. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[4] Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 67. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[5] *Ibid.*, p 68.

[6] Wheeler CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 72-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[7] Wheeler CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p 34. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

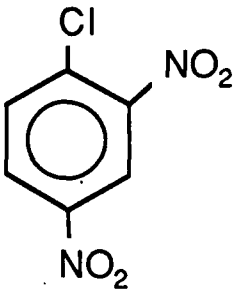
POSITIVE CONTROL

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52–54° C[1]

Purity: The compound was designated as 95% pure by source.

Analytical Data:

Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.[2] Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (d).[3] Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).[4]

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[1] Windholz M, ed. The Merck Index. 10th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

[2] Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3] *Ibid.* pp. 11-12.

[4] *Ibid.* pp. 13-16.

Appendix A (cont.): CHEMICAL DATA

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm^{-1}. The IR spectrum was very close to the Sadtler reference spectrum.[5] Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR ($CDCl_3$): d 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J_{ortho} = 8.7 Hz, J_{meta} = 3.6 Hz), 8.74 (1 H, d, J_{meta} = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.[6] GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses (m/z) of 202 and 204 confirmed the identity of the major peak as DNCB.[7]

Lot Number: 11F-0543

Source: Sigma Chemical Co.
St. Louis, MO

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[5]Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

[6]Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

[7]Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Cavia porcellus*

Strain: Hartley, albino

Source: Charles River Breeding Laboratories
Wilmington, MA

Sex: Male

Date of Birth: 28 December 1984

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 15 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tag.

Pretest conditioning: Quarantine/acclimation 15-29 January 1985

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF EVENTS

Date	Event
15 Jan 85	Animals arrived at LAIR. Animals were examined, weighed, placed in cages, and fed. Animals were assigned ear tags. Two animals were submitted for necropsy quality control.
15 Jan - 1 Mar 85	Animals were checked daily.
21,28 Jan, 4,11,18 25 Feb 85	Animals were weighed.
28 Jan 85	Animals were randomized into three groups (experimental, positive control, negative control) of 15 animals each.
28 Jan, 4,11 Feb 85	Study animals, except negative control group, were clipped and shaved.
29 Jan, 5,12 Feb 85	Study animals, except negative control group, were given induction dose.
30 Jan, 6,13 Feb 85	Study animals, except negative control group , were scored for 24-hr skin reaction.
31 Jan, 7,14 Feb 85	Study animals, except negative control group , were scored for 48-hr reaction.
1,8,15 Feb 85	Study animals, except negative control group , were scored for 72-hr reaction.
25 Feb 85	Study animals were clipped and shaved.
26 Feb 85	Study animals were given challenge dose.
27 Feb 85	Study animals were scored for 24-hr reaction.
28 Feb 85	Study animals were scored for 48-hr reaction.
1 Mar 85	Study animals were scored for 72-hr reaction. All animals were delivered to Necropsy Suite.

Appendix D: INDIVIDUAL ANIMAL SCORES

GROUP: ONE

COMPOUND: TMETN

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										LEFT FLANK			RIGHT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
85E0001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0008	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0027	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0032	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0049	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0061	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0063	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AVERAGES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: TWO

COMPOUND: DNCB

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										LEFT FLANK			RIGHT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
85E0003	0	0	0	1	0	0	0	0	0	1	1	1	1	1	1
85E0005	0	0	0	1	1	0	0	1	0	1	1	1	1	1	1
85E0007	0	0	0	1	1	0	1	1	1	2	1	1	1	1	1
85E0009	0	0	0	1	0	0	1	1	1	2	2	1	1	1	1
85E0014	0	1	0	1	1	1	1	1	0	1	1	1	0	1	0
85E0018	0	0	0	1	0	0	1	1	0	1	1	1	1	0	0
85E0033	0	0	0	1	1	1	1	1	0	1	1	1	0	0	0
85E0035	0	1	0	0	0	0	1	1	1	1	1	1	1	1	0
85E0037	0	0	0	0	0	0	1	1	1	2	1	1	1	1	1
85E0042	0	0	0	1	1	1	1	1	1	2	2	1	1	1	1
85E0050	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2
85E0053	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1
85E0057	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0
85E0060	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1
85E0066	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1
AVERAGES	0.1	0.1	0	0.7	0.5	0.3	0.9	0.9	0.5	1.3	1.2	1.1	0.8	0.9	0.7

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: THREE

COMPOUND: NEGATIVE CONTROL

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										LEFT FLANK			RIGHT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
85E0002	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0011	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0013	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0015	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0023	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0024	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0030	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0039	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0040	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0044	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0045	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0047	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0048	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0052	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
85E0059	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A
AVERAGES	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0	N/A	N/A	N/A

Appendix E: INDIVIDUAL BODY WEIGHTS (grams)

TMETN

Animal Number	DAY OF STUDY							
	Q*0	Q6	Q13	7	14	21	28	32
85E0001	213	281	344	385	426	471	537	510
85E0004	220	273	299	329	376	426	484	455
85E0008	199	250	303	328	364	390	439	416
85E0010	219	270	320	356	396	445	502	471
85E0012	217	277	317	345	381	409	461	439
85E0020	210	287	357	379	435	441	494	475
85E0021	220	281	324	350	378	403	455	423
85E0022	230	293	354	396	454	499	554	530
85E0027	216	271	333	372	426	473	547	504
85E0031	192	252	310	342	383	421	472	440
85E0032	182	234	289	313	348	373	409	384
85E0041	220	274	329	366	420	453	507	469
85E0049	204	269	348	389	437	480	530	506
85E0061	200	232	283	312	347	379	428	399
85E0063	206	279	336	403	447	476	550	504
MEAN	209.9	268.2	323.1	357.7	401.2	435.9	491.3	461.7
Standard Deviation	12.7	18.2	23.0	29.6	35.8	39.6	46.8	44.3
Standard Error	3.3	4.7	6.0	7.6	9.3	10.2	12.1	11.4

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

DNCB

Animal Number	DAY OF STUDY							
	Q*Q	Q6	Q13	7	14	21	28	32
85E0003	213	258	299	318	359	398	445	415
85E0005	216	265	321	341	385	408	455	435
85E0007	216	284	340	378	421	467	533	502
85E0009	211	263	317	353	398	440	508	473
85E0014	198	240	287	321	351	384	429	404
85E0018	223	296	357	386	432	473	515	493
85E0033	196	255	316	360	425	462	406	503
85E0035	202	275	350	406	470	509	606	576
85E0037	193	256	303	339	382	424	476	444
85E0042	191	255	332	370	428	489	553	519
85E0050	219	271	325	356	401	427	478	438
85E0053	198	256	291	311	351	371	403	380
85E0057	206	258	296	316	366	399	434	406
85E0060	215	278	337	382	441	489	540	511
85E0066	216	295	361	408	450	472	520	492
MEAN	207.5	267.0	322.1	356.3	404.0	440.8	486.7	466.1
Standard Deviation	10.4	15.9	23.9	32.0	37.7	42.8	58.9	53.9
Standard Error	2.7	4.1	6.2	8.2	9.7	11.1	15.2	13.9

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

Negative Control

Animal Number	DAY OF STUDY							
	Q*0	06	013	7	14	21	28	32
85E0002	196	251	299	337	382	413	470	466
85E0011	178	233	283	330	381	417	477	450
85E0013	232	281	334	371	423	468	518	491
85E0015	201	258	314	336	365	400	438	403
85E0023	191	253	304	346	384	431	472	450
85E0024	221	295	359	408	478	525	583	553
85E0030	194	268	320	384	451	513	583	553
85E0039	189	234	292	340	388	433	479	449
85E0040	198	264	325	380	437	508	580	551
85E0044	182	231	288	332	367	391	435	412
85E0045	222	285	342	390	459	493	526	509
85E0047	221	278	325	370	425	463	525	494
85E0048	199	268	331	384	432	470	524	498
85E0052	222	295	346	400	462	512	557	555
85E0059	220	290	353	422	490	539	580	553
MEAN	204.4	265.6	321.0	368.7	421.6	465.1	516.5	492.5
Standard Deviation	17.0	22.0	24.0	30.1	41.5	48.7	52.6	53.0
Standard Error	4.4	5.7	6.2	7.8	10.7	12.6	13.6	13.7

* Q represents quarantine period.

Appendix F: PATHOLOGY REPORT

LAIR Pathology Report
GLP Study 84Ø42
Buehler Dermal Sentization, TMETN
in Guinea Pigs

History: Forty-five male Hartley Albino guinea pigs were divided into three groups of 15 each. Experimental, Positive control, and Negative control, and tested in accordance with LAIR SOP OP-STX-82. Live animals were submitted to necropsy, killed with an overdose of sodium pentobarbital and examined grossly. Selected skin samples were taken as well as liver specimens from two animals.

Gross Pathology Results

Path #	Group	Animal #	Findings
36966	experimental	85EØØØØ1	Hepatic necrosis, multifocal, minimal*
36967	experimental	85EØØØØ4	Hepatic necrosis, multifocal, mild*
36968	experimental	85EØØØØ8	Hepatic necrosis, multifocal, mild
36969	experimental	85EØØØ1Ø	Hepatic necrosis, multifocal, mild
3697Ø	experimental	85EØØØ12	Hepatic necrosis, multifocal, mild
36971	experimental	85EØØØ2Ø	Hepatic necrosis, multifocal, moderate
36972	experimental	85EØØØ21	Hepatic necrosis, multifocal, moderate
36973	experimental	85EØØØ22	Hepatic necrosis, multifocal, severe
36974	experimental	85EØØØ27	Hepatic necrosis, multifocal, mild
36975	experimental	85EØØØ31	Hepatic necrosis, multifocal, mild
36976	experimental	85EØØØ32	Hepatic necrosis, multifocal, minimal Ø.5 cc yellow fluid thorax
36977	experimental	85EØØØ41	Liver necrosis, multifocal, moderate
36978	experimental	85EØØØ49	Hepatic necrosis, multifocal, moderate
36979	experimental	85EØØØ61	Hepatic necrosis, multifocal, mild
3698Ø	experimental	85EØØØ63	Hepatic necrosis, multifocal, moderate

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84042

Path #	Group	Animal #	Findings
36981	positive	85E00003	Hepatic necrosis, multifocal, mild
36982	positive	85E00005	Hepatic necrosis, multifocal, mild
36983	positive	85E00007	Hepatic necrosis, multifocal, moderate
36984	positive	85E00009	Hepatic necrosis, multifocal, mild
36985	positive	85E00014	Hepatic necrosis, multifocal, minimal
36986	positive	85E00018	Hepatic necrosis, multifocal, mild
36987	positive	85E00033	Hepatic necrosis, multifocal, mild
36988	positive	85E00035	Hepatic necrosis, multifocal, minimal
36989	positive	85E00037	Hepatic necrosis, multifocal, moderate
36990	positive	85E00042	Hepatic necrosis, multifocal, mild
36991	positive	85E00050	Hepatic necrosis, multifocal, moderate
36992	positive	85E00053	Hepatic necrosis, multifocal, minimal
36993	positive	85E00057	NR (Not Remarkable)
36994	positive	85E00060	Hepatic necrosis, multifocal, mild
36995	positive	85E00066	NR
36996	negative	85E00002	Hepatic necrosis, multifocal, minimal
36997	negative	85E00011	Hepatic necrosis, multifocal, mild
36998	negative	85E00013	Hepatic necrosis, multifocal, minimal
36999	negative	85E00015	NR

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84042

Path #	Group	Animal #	Findings
37000	negative	85E00023	Hepatic necrosis, multifocal, minimal
37001	negative	85E00024	Hepatic necrosis, multifocal, minimal
37002	negative	85E00030	Hepatic necrosis, multifocal, moderate
37003	negative	85E00039	Hepatic necrosis, multifocal, minimal
37004	negative	85E00040	Hepatic necrosis, multifocal, moderate
37005	negative	85E00044	Hepatic necrosis, multifocal, minimal
37006	negative	85E00045	Hepatic necrosis, multifocal, minimal
37007	negative	85E00047	Hepatic necrosis, multifocal, minimal
37008	negative	85E00048	Hepatic necrosis, multifocal, mild
37009	negative	85E00052	Liver, pale brown
37010	negative	85E00059	Hepatic necrosis, multifocal, mild

*Microscopic examination of liver done

Histopathology Results Skin:

36966: Two slides, four tissues - NR

36967: Two slides, four tissues - NR

36996: Two slides, four tissues - NR

36997: Two slides, four tissues - NR

36981: Two slides, four tissues - NR

36982: Two slides, four tissues - NR

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 84042

Histopathology Results of Liver:

36966 - Slide 3, two sections of liver: There were several small foci of hepatocellular coagulative necrosis lined by a few inflammatory cells. Other small areas were present in which the hepatocytes were lost and the stroma had collapsed but the inflammatory cell border was still there. One focus had bile duct hyperplasia as well.

36967 - Slide 3, three sections of liver: There were several moderate to large foci of hepatocellular necrosis and or degeneration. Some of these areas were lined and infiltrated by inflammatory cells and others had few inflammatory cells. There was stromal collapse and hepatocellular regeneration in some areas along with bile duct hyperplasia.

Comments: There were no lesions seen that could be attributed to the test compound. The hepatocellular necrosis seen was considered to be incidental to the compound and most likely due to the repeated handling of these guinea pigs. This is not uncommon in this species. The variability of the age of these lesions supports this interpretation.

LANCE O. LOLLINI, DVM
LTC, VC
Chief, Pathology Services Group

Distribution List

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Cameron Station
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US Army Medical Research and Development Command (2)
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